
Sophie Trouillet-Assant,a,b,c Michele Bes,a,b,c Helene Meugnier,a,b,c Sylvestre Tigaud,a Jérôme Etienne,a,b,c François Vandenbossche,a,b,c Frédéric Laurenta,b,c

Department of Clinical Microbiology, Hospices Civils de Lyon, Lyon, France; National Reference Center for Staphylococci, Lyon, France; International Center of Infectious Research, INSERM U1111, CNRS UMR5308, University of Lyon 1, ENS de Lyon, Lyon, France

Using a large collection of European and North African methicillin-resistant Staphylococcus aureus (MRSA) isolates with a variety of genetic backgrounds and staphylococcal cassette chromosome mec (SCCmec) types, we evaluated the reliability of the BD GeneOhm MRSA assay. Results revealed high performance of this test for detecting MRSA strains provided from Europe and North Africa (98.3%).

Methicillin-resistant Staphylococcus aureus (MRSA) is one of the major pathogens responsible for nosocomial infections (1). Early screening for MRSA carriage in patients is essential for limiting transmission (2). To reduce the long response delay with conventional cultures (approximately 48 to 72 h), real-time PCR assays have been developed to ensure the rapid and reliable detection of MRSA. The BD GeneOhm MRSA assay (BD Diagnostics GeneOhm, Quebec City, QC, Canada) is a molecular test that detects a specific genetic fragment in the staphylococcal cassette chromosome mec (SCCmec) right extremity junction (MREJ), as described by Huletsky et al. (3). MRSA strains are not only derived from a variety of genetic backgrounds but they also present a high diversity of SCCmec elements which are characterized by the mec class, ccr recombinase complex, and three “joining regions.” Eleven SCCmec types have been described based on the different combinations of these genetic structures, and they are referenced by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC) (http://www.sccmec.org/). Several studies have endeavored to assess the reliability of this test for detecting MRSA strains by using collections of genotypically diverse MRSA strains. Notably, Boyle-Vavra and Daum have tested 914 MRSA isolates from the United States and Taiwan (4). The genetic backgrounds of MRSA strains are different between continents because of the geographical spread of specific clones (5); therefore, the aim of this study was to assess the reliability of the BD GeneOhm MRSA assay using a large collection of genetically diverse MRSA isolates from Europe and North Africa.

A total of 1,000 MRSA isolates collected between 2002 and 2010 that originated from Western, Central, and Eastern Europe and North Africa were tested. Isolates from the collections stored in the National Center of Staphylococci (Lyon, France) were selected to maximize the diversity of the genetic backgrounds and SCCmec types. The isolates were distributed as follows: Algeria, n = 42; Germany, n = 60; Austria, n = 41; Belgium, n = 51; Bulgaria, n = 10; Croatia, n = 10; Denmark, n = 47; Spain, n = 48; Finland, n = 9; France, n = 122; Greece, n = 18; Netherlands, n = 29; Hungary, n = 6; Ireland, n = 26; Israel, n = 29; Italy, n = 61; Poland, n = 26; Portugal, n = 16; Russia, n = 40; Senegal, n = 29; Slovenia, n = 28; Sweden, n = 4; Switzerland, n = 68; Czech Republic, n = 12; Togo, n = 3; Tunisia, n = 48; Turkey, n = 21; and the United Kingdom, n = 42. In addition, 54 strains isolated from pigs in France, Denmark, and Belgium were also tested. SCCmec typing was performed using Kondo’s method and DNA microarrays (6, 7). MRSA suspensions were grown to a turbidity of a 0.5 McFarland standard. Fifty microliters of suspension was added to a lysis tube containing small glass beads, and the suspension was vortexed to lyse the bacteria. The BD GeneOhm MRSA assay was then performed using a SmartCycler (Cepheid, Sunnyvale, CA, USA) according to the manufacturer’s instructions, and both a positive control and a negative control were included in each run. Three additional replicates using the same lysate were performed to confirm isolates that had tested negative using the BD GeneOhm MRSA assay. Furthermore, to confirm the integrity of these lysates, an in-house PCR (specific to S. aureus and the mecA gene) was performed as previously described (8). Isolates that tested negative using the BD GeneOhm MRSA assay and positive using the in-house PCR test were considered to be false negatives for the commercial assay. In addition, all of the false negatives from the BD GeneOhm MRSA assay were extensively characterized using an S. aureus-specific diagnostic DNA microarray (StaphyType; Clondiag, Jena, Germany) that covers 330 S. aureus-specific sequences (6, 7). The affiliation of isolates with clonal complexes (CCs) was determined by comparing their hybridization profiles with the reference strains included in the StaphyType database. spa typing and based upon repeat pattern (BURP) analysis were also performed as previously described (http://spaserver.ridom.de).

Of the 1,000 genotypically diverse isolates from Europe and North Africa used in this study, 983 tested positive (98.3%) using the BD GeneOhm MRSA assay. These results are consistent with those obtained by Boyle-Vavra and Daum (99.7%) using 914 iso-
lates from a large collection of genetically diverse isolates from the United States and Taiwan (4). Taken together, these results confirm that the BD GeneOhm MRSA assay has a high capacity to detect MRSA clones from diverse genetic backgrounds, including geographically diverse strains from Europe and North Africa.

Only 17 of the isolates (1.7%) were not detected as MRSA (Table 1), and all of these strains were confirmed to be positive for mecA using an in-house PCR. All of these nontected isolates originated in Western Europe (Belgium, Denmark, France, Finland, Netherlands, Portugal, and Switzerland). The BURP analysis revealed that 35% (6/17) of these isolates clustered into the same spa group (spa CC024), which includes the spa types t008, t024, and t068. Interestingly, Bartels et al. evaluated the performance of the BD GeneOhm MRSA assay using a Danish collection of MRSA isolates that were primarily from Copenhagen and reported that 12.6% (44/349) were false negatives (9). The most common clone among the false negatives in the Danish study harbored the spa type t024 (sequence type [ST8]-SSCMec IVa), which belongs to the spa group CC024 (data not shown). Taken together, these results suggest that a subset of the strains misidentified by the BD GeneOhm MRSA assay are phylogenetically related. Nevertheless, certain true-positive isolates (harboring spa types t051, t112, t121, t190, t451, t622, t801, t1635, t1677, t2054, t2206, t2942, t3060, and t4146) also belonged to the spa group CC024. These data suggest that the spa type or spa CC should not be used as the sole determinant for characterizing the genetic backgrounds of the false-negative MRSA strains by the BD GeneOhm MRSA assay.

Zhang et al. characterized the SCCmec elements in the BD GeneOhm MRSA assay-negative strains and suggested that this type of misidentification can be due to the insertion of non-mec-containing SCC elements downstream of the orfX gene and carriage of SCCmec in the left extremity, which is not detectable using this molecular MRSA test (10). In our study, most of the false-negative MRSA isolates (10/17; 59%) harbored a composite SCCmec type according to the microarray analysis of additional ccr genes (Table 1). For the remaining strains, the presence of other insertional cassettes (for which ccr genes are not detected by microarrays) or SCCmec in an atypical extremity is highly likely.

García-Álvarez et al. have recently reported the emergence of a highly divergent mecA homologue, mecC, which is located in a novel SCCmec element, named SCCmec type XI (11). These strains combine an unrecognized mec gene with an unrecognized SCCmec cassette, and mecC has only a 70% identity at the DNA level with other known mecA homologues (11, 12). Genome analysis revealed that three final open reading frames (ORFs) are located downstream of SCCmec XI and are considered to be remnants of an SCC element. Such MRSA strains would most likely be falsely identified as methicillin-susceptible Staphylococcus aureus (MSSA) strains because the primers supplied in the MRSA detection kit are not able to recognize the new divergent target sequences of the SCCmec cassette.

Overall, our results demonstrate that the BD GeneOhm MRSA assay provides accurate detection of most of the MRSA strains circulating in Europe and North Africa (98.3%). Nevertheless, our study highlights the importance of performing regular sentinel studies in different parts of the world to rapidly identify the emergence and dissemination of new MRSA clones and incorporate new oligonucleotide sequences into this test to keep up with the dynamic epidemiology of Staphylococcus aureus species.

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REFERENCES


